

Listing of Claims:

1. (Currently amended): A fluorescence polarization protein binding assay for measuring inositol 1,4,5-triphosphate (IP₃) in a sample employing as reagents a conjugate of IP₃, a fluorescent label joined through – R – Z -, wherein R is a bond or linker at the 2-hydroxyl position of said IP₃, R is of not more than 16 atoms in the chain and Z is selected from the group consisting of oxy, amino, thio, succinimidyl, amino, ureido, ester, phospho, thiophospho, and oxalo, and as a binding protein a fusion protein of up to 1.5k of amino acids and comprising 226 – 578 amino acid extracellular portion of mouse inositol 1,4,5-triphosphate receptor (IP₃R) having at least about 200 times the affinity for IP₃ than the intact IP₃R, wherein said conjugate and IP₃ in the sample compete for binding to said binding protein and the amount of bound or unbound conjugate will be related to the number of binding proteins bound by IP₃ in said sample, said method comprising:

combining in an assay medium said sample, wherein said sample is a cellular lysate and said cellular lysate has been treated to block kinases and phosphatases, said conjugate and said binding protein and incubating said mixture for sufficient time for complex formation of IP₃ and said conjugate with said binding protein; and

detecting the bound or unbound label as a measure of the IP₃ present in the sample.

2. (Original): A protein binding assay according to Claim 1, wherein said assay is in a homogeneous format.
3. (Original): A protein binding assay according to Claim 1, wherein said sample is a cellular lysate, and wherein said cellular lysate has been treated to block kinases and phosphatases and prepare said sample for said assay.

4. (Previously Presented): A protein binding assay according to Claim 1, wherein said binding protein is of not more than about 600 amino acids and comprises at least amino acids 226 – 578 of the mouse IP₃R Type 1 fused to glutathione-S-transferase.
5. (Canceled).
6. (Canceled).
7. (Previously presented): A protein binding assay according to Claim 1, wherein said label is 2-O- (2-aminoethyl- (6-carboxamidofluoresceinyl).
8. (Original): A method according to Claim 1, wherein the order of addition of reagents is: (a) combining said sample with said binding protein; and (b) adding said conjugate, with incubating after (a) and (b).
- 9 -21. (Canceled).